S-Nitrosylation of m2 Muscarinic Receptor Thiols Disrupts Receptor–G-Protein Coupling[a]

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Muscarinic acetylcholine receptors possess nine cysteine residues which are conserved among all five receptor subtypes: four are in membrane-spanning helices; one is in the cytoplasmic NH_2-terminal tail and is probably palmitoylated; two are in the third extracellular loop; and two cysteines located in the first and second extracellular loops participate in disulfide bond formation.[1,2] In addition, each receptor has a variable number (1–6) of cysteine residues in the large third intracellular loop. Although biochemical manipulation of these groups has little effect on [^{3}H]antagonist binding, it has been known for at least 10 years that the redox state of these sulfhydryl moieties influences the functional coupling of receptors to transducer G-proteins.[3,4] However, natural mediator(s) that may act on these groups to regulate receptor activity have not been identified. The work of Lipton and co-workers[5] raises the possibility that *S*-nitrosylation of reactive sulfhydryl centers may be a common pathway for molecular control of protein function. Nitric oxide (NO) group transfer (of NO^+) from nitric oxide depends on the redox chemistry intrinsic to the NO molecule.[5] Sodium nitroprusside (SNP) has a strong NO^+ character and nitrosylates protein thiols. In the present study, we examined the possibility that nitrosylation of sulfhydryl groups by NO^+ affects receptor–G-protein coupling.

Rat atrial membranes contain m2 muscarinic receptors whose binding of [^{3}H]*N*-methylscopolamine increases dramatically in the presence of Gpp(NH)p and after physical treatments (heat, low pH) that engender m2 dissociation from G-proteins.[4,6] Exposure of atrial m2 receptors to SNP (1–5 mM for 30 minutes at room temperature) produced a moderate (18–30%) increase in [^{3}H]MS binding (FIG. 1A). Gpp(NH)p (10 μM) increased the apparent density of muscarinic binding sites by 60% (FIG. 1B). However, marked stimulation of [^{3}H]MS binding by Gpp(NH)p was moderated by SNP (FIG. 1B). Fully two-thirds of the increase in binding engendered by Gpp(NH)p was eliminated by 5 mM SNP, so that [^{3}H]MS binding was reduced to a level lower than that measured after exposure to 5 mM SNP alone. Under redox conditions (i.e., exposure to ascorbate) which promoted $NO\cdot$, as opposed to NO^+, formation from SNP, m2 binding and activity were severely depressed or eliminated

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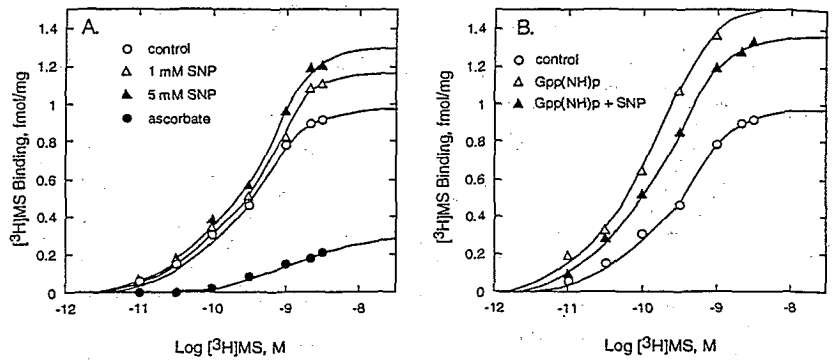

FIGURE 1. Influence of sodium nitroprusside (SNP) on the binding of [^{3}H]MS to atrial m2 receptors. Each point represents the mean from 3–6 experiments. (A) The binding of the indicated concentrations of [^{3}H]MS was measured in the absence of SNP (○) or after exposure to 1 (△) or 5 (▲) mM SNP. In one series of experiments, atrial membranes were treated with 1 mM ascorbate for 10 min at room temperature before being exposed to 1 mM SNP (●). Nonlinear regression analyses using a single receptor population model indicated no change in [^{3}H]MS affinity but increases in apparent receptor density from 0.99 ± 0.07 to 1.18 ± 0.12 and 1.30 ± 0.16 fmol/mg protein after exposure to 1 and 5 mM SNP, respectively. (B) The binding of [^{3}H]MS was measured in the absence of modulating agents (○) or in the presence of 10 μM Gpp(NH)p (△) or 10 μM Gpp(NH)p after exposure to 1 mM SNP (▲). Nonlinear regression analyses using a single receptor population model indicated an increase in receptor density in the presence of Gpp(NH)p (from 0.99 ± 0.07 to 1.57 ± 0.05 fmol/mg protein) with no change in [^{3}H]MS binding affinity. The apparent receptor density in the presence of Gpp(NH)p was reduced to 1.39 ± 0.11 and 1.19 ± 0.08 after exposure to 1 (▲) and 5 (not shown) mM SNP, respectively.

(FIG. 1A). Exposure to ascorbate alone did not alter [^{3}H]MS binding. Sodium nitroprusside (5 mM) decreased surface and total thiol content by 27% with an IC_{10} of 1.0 ± 0.2 mM (n = 3) (FIG. 2). These effects of SNP were not observed under conditions (i.e., heat and low pH) in which receptor–G-protein coupling was eliminated. Thus, *S*-nitrosylation appears to uncouple m2 receptors from transducer.

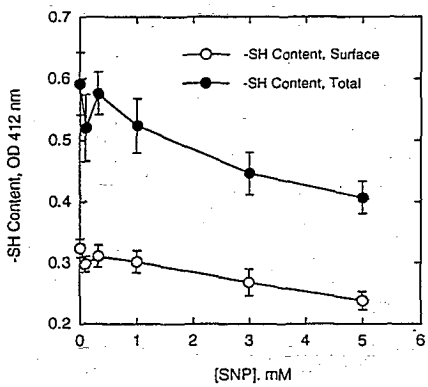

FIGURE 2. Influence of sodium nitroprusside (SNP) on the sulfhydryl content of atrial membranes. Sulfhydryl content was measured after a 10-min exposure to SNP in undisrupted membranes (○) or in SDS-solubilized (●) membranes. Measurements were performed spectroscopically (412 nm) using 5.5′-dithio-*bis*-2-nitrobenzoate as a specific probe.

G-proteins. It is interesting that SNP effects were not observed in cultured human neuroblastoma cells (Sk-N-SH), which predominantly express m3 muscarinic receptors. Preliminary experiments also indicate that treatment with 5 mM SNP disrupts m2 receptor control of G-protein GTPase activity, although the nature of this effect (i.e., a receptor, G-protein, or coupling effect) is not yet clear.

These findings suggest a mechanism for modulation of muscarinic receptor function that depends on both the redox milieu and nitric oxide production. Although the identity of the relevant reactive sulfhydryl center awaits confirmation by mutational analysis, the fact that m2 but not m3 receptors are selectively affected might indicate involvement of the cysteine moiety on the second intracellular loop, which is only present in m2 and m4 receptors. This work supports the suggestion by Lipton and co-workers[5] that nitrosylation of reactive sulfhydryl groups is a common biochemical mechanism for the control of protein function.

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